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(19) (CA) **CANADIAN PATENT** (12)

(54) Cosmetics and Pharmaceuticals Containing Extensins and
Related Methods

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ABSTRACT OF DISCLOSURE

Cosmetic and pharmaceutical compositions containing effective amounts of extensin proteins and the related methods.

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COSMETICS AND PHARMACEUTICALS CONTAINING EXTENSINS
AND RELATED METHODS

Technical Field

The invention is in the field of cosmetic and pharmaceutical compositions containing extensin proteins.

Background of the Invention

Cosmetics companies are particularly interested in formulating treatment cosmetics which exert beneficial effects on skin and hair. Since it is known that various animal
10 derived substances provide beneficial effects to skin and hair, it has become popular to incorporate these substances into cosmetics and personal care products. For example, animal collagen is known to have moisturizing and film forming properties, and is a popular additive to treatment cosmetics. Animal collagen protein is the main component of connective tissues, skin, muscles and tendons. It is a fibrous protein of about 100,000 molecular weight, rich in proline and hydroxyproline, and structurally analogous to a three-stranded rope in which each strand is a polypeptide chain. Collagen is
20 responsible for most of skin structure. In the course of aging the polypeptide chains of collagen polymerize. The result is "cross linking", which causes wrinkling of the skin as well as reduction in skin elasticity.

Other non-collagen animal proteins such as plasma proteins, placental proteins, or proteins from milk sources are



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also popular as cosmetics additives, as well as proteins from lower animals such as silkworm, fish, bacteria, yeast, or non-specified marine sources.

Extensins are a family of plant derived hydroxyproline rich glycoproteins (HRGP) firmly bound to the primary cell wall of several species of monocotyledonous and dicotyledenous plants. Extensins are also rich in serine, valine, tyrosine, lysine, and in some instances threonine, and the polypeptide backbone comprises repeating hydroxyproline units in
10 conjunction with other basic amino acids such as valine, lysine, proline, tyrosine, histidine, serine, and threonine. The hydroxyproline component is heavily glycosylated. Extensins play a role in growth, regulation, stress response, cell-cell recognition, and reproductive physiology of plants. The protein is widely distributed throughout the plant kingdom. Extensins are generally insoluble in muro because of their extensive cross linking, so the intact protein has not been isolated from mature plants, which has made the scientific study of intact extensin protein much more difficult. A
20 hydrolyzed form of extensin is available commercially through Centerchem, Stamford, Connecticut. However, this hydrolyzed product contains very small polypeptide chains having molecular weights of 100-1500 daltons. The estimated size of intact extensin protein is greater than 100,000 daltons, or about 100,000-150,000 daltons.

It is known that prior to incorporation of insoluble cross linked extensin into the plant cell wall it exists in a soluble form which can be isolated by salt extraction of cell

suspension cultures containing the soluble precursor form.

It has unexpectedly been discovered that this soluble form of extensin may be incorporated into cosmetics as an analog for animal collagen and will act to smooth, tighten, and enhance skin texture. Extensins also contain large amounts of humectant sugars which are capable of binding water, thus making them ideal humectants.

Summary of the Invention

10 The invention is directed to cosmetic compositions comprising cosmetically effective amounts of extensin protein.

The invention is directed to pharmaceutical compositions comprising pharmaceutically effective amounts of extensin protein.

The invention is directed to a method of moisturizing and forming a film on skin comprising applying to the skin a cosmetic or pharmaceutical composition containing an effective amount of extensin protein.

Description of Drawings

20 Figure 1 shows a scanning electron micrograph of a silicone replica of skin at 25X magnification. About 40 microliters of a 0.2% solution of extensin from carrots was applied to the right half of an area 15 millimeters in diameter (about 44 square millimeters). The left half of the area was left untreated.

Figure 2 shows a scanning electron micrograph of a silicone replica of skin at 25X magnification. About 40 microliters of a 0.2% solution of a high molecular weight

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non-extensin protein from wheat was applied to the right half of an area 15 millimeters in diameter (44 square millimeters of treated surface area), while the left half was left untreated. A comparison of both figures reveals that skin treated with extensin protein has an appreciably more intact and visible film than skin treated with high molecular weight non-extensin wheat protein or untreated skin.

Detailed Description

10 The term "extensin" or "extensin protein" means a soluble hydroxyproline-rich glycoprotein analog to animal collagen which is extracted from plant cell walls. Structurally extensins are comprised of a polypeptide backbone comprising repeating hydroxyproline units in conjunction with other basic amino acids such as valine, lysine, proline, tyrosine, histidine, serine, and threonine. The term "extensin" may mean extensin hydrolysates wherein the molecular weight of the fragments is from 100 - 100,000 daltons, or more likely 100-1500 daltons; or it may mean the intact extensin protein which has a molecular weight of about 100,000 daltons up to
20 150,000 daltons.

The extensin hydrolysates may be purchased from Centerchem, Stamford Connecticut, and exhibit molecular weights of 100-1500 daltons.

The intact extensin proteins used in the cosmetic compositions of the invention are described in the following publications and may be extracted from various plants by methods described in these publications: Biochimica Et

Biophysica Acta, Vol. 257(1972)421-432; Plant Physiology, Vol. 76 (1984), 414-417; Phytochemistry, Vol. 23, No. 6 (1984) 1233-1239; Plant Physiology Vol. 85 (1987) 823-827; Plant Physiology, Vol. 87 (1987) 616-621; Plant Physiology, Vol. 86 (1988) 848-856; Phytochemistry, Vol. 25, No. 5 (1986) 1021-1030.

10 Generally, soluble extensin precursors may be extracted from plant species in the growth phase by salt extraction of crude extensin protein fractions from concentrated plant cell suspension pellets or explant homogenates. Various metallic salt solutions are suitable for this purpose including aluminum chloride, calcium chloride, lapyrium chloride, sodium chloride, etc. The crude extensin protein extracts may then be further purified by acid precipitation of contaminants using acids such as trichloroacetic acid. Extensin proteins are generally soluble in 5-10% trichloroacetic acid. Then standard protein purification methods may be used for further purification, for example size exclusion affinity chromatography or ion exchange chromatography.

20 It is preferred to utilize the intact extensin protein (of greater than about 100,000 daltons molecular weight) in the cosmetics of the invention, although the use of extensin hydrolysates of small molecular weight fragments of 100-1500 daltons are suitable also.

A 0.5% solution of extensin generally produces a flexible film that keeps the skin pliable and firm for five to six hours. The cosmetic benefits are seen in cosmetic and

pharmaceutical compositions as outlined below.

The extensin extracts may be incorporated into a variety of cosmetic and pharmaceutical compositions. Cosmetic or pharmaceutical compositions containing effective amounts of extensins improve texture, smoothness, and moisture content of the skin.

The term "cosmetic composition" means a composition applied externally to skin, nails, or hair of the human body, for purposes of beautifying, coloring, conditioning, or protecting the bodily surface. Examples of cosmetic compositions in accordance with the invention include lotions, creams, moisturizers, gels, sun screens, makeup, cleansers, shampoos, hair conditioners, skin firming compositions, protein concentrates, after shaves, eyeshadows, blushes, nail enamels and so on.

The term "pharmaceutical composition" means a composition applied externally to the skin, hair, or nails of the human body for therapeutic purposes. Examples of pharmaceutical compositions in accordance with the invention include ointments, creams, lotions, gels, solutions, and so on.

The invention comprises cosmetic compositions comprising a cosmetically effective amount of extensin protein. A cosmetically effective amount of extensin protein in accordance with the invention is about 0.01-30% by weight of the total composition, with 0.1-10% preferred, and 0.5-5% most preferred.

The extensin proteins may be incorporated into a wide

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variety of cosmetic compositions. For example, cosmetically effective amounts of extensin may be incorporated into moisturizing lotions for application to human facial or body skin. These lotions generally contain from about 20-80% oil and 10-80% water in an emulsion form. In addition the moisturizing lotion may contain humectants, emollients, surfactants, fragrances, preservatives, and so forth. About 5-10% humectant, about 5-20% emollient, and about 0.5-10% surfactant are suggested.

10 Extensins may be easily incorporated into moisturizing creams. Creams generally contain from about 20-70% water and about 30-70% oil. In addition, creams may contain a variety of humectants, emollients, surfactants, preservatives, and fragrances. About 5-10% humectant, about 5-20% emollient, and about 0.5-10% surfactant are suggested.

Extensins may also be incorporated into treatment makeups. Generally makeup formulations comprising 5-70% oil, 10-95% water, and about 5-40% pigment are suitable. In addition the makeup may contain surfactants, silicone, humectants, emollients, preservatives, fragrances, etc.
20 Generally 0.5-10% surfactant, 0.1-30% silicone, 5-10% humectant, 0.1-30% emollient, and 0.1-5% preservative is suggested.

Extensins may also be incorporated into colored cosmetics such as eyeshadow or blush. For example, a suitable eyeshadow comprises 5-40% pigments, 1-50% oil, and 1-20% waxes. . Additionally the composition may contain one or more of 10-60% water, 0.5-30% surfactant, 1-10% humectants, 0.1-5%

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preservative, and 0.1-20% silicone.

Extensins are also suitable for incorporation into shampoos and hair conditioners. Suitable shampoo formulations include 1-40% surfactant and 10-90% water. Suitable hair conditioning formulations include 30-95% water, 0.5-30% conditioning ingredients such as emollients, proteins, shine enhancers, and so forth, and 1-40% surfactant. Hair conditioners and shampoos may also contain thickeners and silicone. About 0.05-15% silicone is suggested in shampoos and hair conditioners.

Extensins may also be incorporated into cleansers, aftershaves, toners, fragrance splashes, and even nail treatment products or nail enamels. For example, fragrance splashes, aftershaves and toners, generally comprise about 10-70% alcohol. In addition, 0.01-5% surfactant may be added as well as 1-5% humectants, and up to 25% perfume.

Extensins may be incorporated into traditional nail enamels or nail treatment products which generally comprise about 1-40% film former, 10-50% resin, and 10-70% solvent, in addition to the usual plasticizers, pigments, and wetting agents.

The invention is also directed to pharmaceutical compositions comprising pharmaceutically effective amounts of extensin protein.

A pharmaceutically effective amount in accordance with the invention means about 0.01-30% of extensin protein, with 0.1-10% preferred, and 0.5-5% most preferred. The extensin

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proteins may be incorporated into suitable pharmaceutical vehicles such as lotions, creams, ointments, gels, or solutions. Suitable ointments are hydrophilic ointments (USP) or petrolatum and cosmetically effective amounts of extensin protein are incorporated into the ointment for topical application to skin. Suitable lotions and creams are as mentioned previously for cosmetic compositions. Solutions are made by mixing solutions of extensin protein in deionized water for application to human skin. Gels are made by mixing 1-90%
10 water with a suitable polymer.

Suitable humectants for use in the cosmetic compositions of the invention include glycerin, propylene glycol, butylene glycol, urea, sorbitol, sodium PCA, gelatin, polyethylene glycols, sodium lactate, hyaluronic acid, and so on.

Suitable emollients include glyceryl stearate, cetyl alcohol, stearyl alcohol, isopropyl stearate, stearyl alcohol, stearyl stearate, isopropyl stearate, stearic acid, isobutyl palmitate, isocetyl stearate, oleyl alcohol, sebacates, myristates, palmitates, squalenes, glyceryl monooleate, oleic
20 acids, lanolin, acetylated lanolin alcohols, petrolatum, mineral oils, palmitic acids, isostearyl neopentanoate, etc.

A variety of surfactants may be used in the compositions of the invention including amphoteric, anionic, cationic or nonionic surfactants. Suitable amphoteric surfactants include imidazolines, betaines, and amino acid salts. Suitable anionic surfactants include fatty acid soaps, salts of higher alkyl sulfates, n-acyl sarcosinates, salts of phosphates,

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sulfosuccinate salts, alkyl benzene sulfonates, salts of N-acyl glutamate, polyoxyethylene alkyl ether carboxylic acids, and so on. Cationic surfactants include alkyl trimethyl ammonium salts, alkyl pyridinium salts, alkyl quaternary ammonium salts, polyamine fatty acid derivatives, etc. Nonionic surfactants include lipophilics such as sorbitan fatty acid esters, glycerol fatty acids, propylene glycol fatty acid esters; hydrophilics such as polyoxyethylene sorbitan fatty acid esters, polyoxyethylene glycerol fatty acid esters, 10 polyoxyethylene fatty acid esters, polyoxyethylene alkyl ethers, pluronics, polyoxyethylene alkyl phenyl ethers, polyoxyethylene propylene glycol fatty acid esters, and so on.

Suitable pigments include organic and inorganic pigments such as talc, mica, titanium dioxide, titanated mica, iron oxides, ultramarines, chromium oxides, carmine, D&C and FD&C colors and lakes, ferric and ferrous oxides, and so on.

Suitable preservatives include imidazolidinyl urea, the parabens, quaternium 15, benzyl alcohol, phenoxyethanol and so on.

20 Suitable waxes include beeswax, carnauba, ceresin, microcrystalline, lanolin, paraffin, ozokerite, lanolin alcohol, acetylated lanolin, candelilla, cetyl alcohol, cocoa butter, petrolatum, hydrogenated castor oil, spermaceti, bran wax, capok wax, bayberry, etc.

The invention is also directed to a method for moisturizing and forming a film on human skin, nails, or hair comprising applying to the surface an effective amount of

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extensin protein. An effective amount of extensin is about 0.01-30% by weight. The extensin protein may be applied directly to the surface in a solution form, or it may be incorporated into the cosmetic or pharmaceutical compositions mentioned herein. The extensin protein or protein containing composition may be applied to the surface once or twice a day or as necessary. For example, if the extensin protein is incorporated into a facial moisturizer, usually one to two applications of moisturizer per day will provide a beneficial effect. If the extensin proteins are incorporated into shampoos or hair conditioners, usually application once a day or every other day will be sufficient to provide a beneficial effect. When extensins are incorporated into makeups, blushes, or eyeshadows, they provide a treatment effect to the skin when applied once a day or whenever makeup is worn. If incorporated into nail treatment products or nail enamels, consistent usage in a nail care regimen (i.e. once or twice a week) will provide beneficial results.

The invention will be further described in connection with the following examples which are set forth for the purposes of illustration only.

Example 1

Bean seeds, (*Phaseolus Vulgaris*) or carrots (*Daucus carota*) are purchased from local gardening stores and the seeds germinated on moist filter paper in the dark. Germinated seeds are then planted in large window boxes or in gardens. Young plants are grown and then the hypocotyl and roots harvested and homogenized in 50 mM potassium phosphate buffer pH 6, 1 mM dithiothreitol (DTT), 0.1 mM phenyl methylsulfonyl fluoride and

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2 mM ascorbate in an Osterizer (Waring) blender. The homogenate was diluted with 10 mM calcium chloride and centrifuged at 1000 x G. Pellets were extracted again in 100 mM calcium chloride and recentrifuged. The supernatants were combined and dialyzed to remove excess salt. The result was a crude extensin extract suitable for incorporation into cosmetics or pharmaceuticals.

Example 2

Small tomato plants are obtained from local gardening stores and acclimated to outdoor gardens. After two weeks small amounts of plant tissue are cut off and frozen in liquid nitrogen until a sufficient amount is collected (approximately 100 grams of wet tissue). Crude homogenates are made and extracted with 75 mM aluminum chloride.

Example 3

Small pieces of potato are rooted by exposing the pieces to water containing 35 mM sodium azide, 0.5% ferulic acid, 0.05% curcumin, 0.1% carnosine, or 0.05% extract from spinach, gingo bilboa, or ginseng. The root systems formed are harvested and homogenized in 50-100 mM calcium chloride or 150 mM sodium chloride.

Example 4

Pea seeds (Pisum sativum) are germinated and the epicotyl and root sections were dissected and extracted in a loosely stoppered flask at 70° C. with 0.12% v/v acetic acid and 0.3% sodium chlorite for 30 minutes under a nitrogen blanket. After cooling the solution was filtered through coarse glass wool and

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the residue washed five times with distilled water. Nitrogen is bubbled through the filtrate to remove any extraneous chlorine dioxide. The resulting homogenate is dialyzed to yield a crude extensin protein preparation.

Example 5

An extensin containing oil in water moisturizing lotion is made as follows:

	<u>w/w%</u>
Glyceryl stearate	3.0
10 PPG-10 lanolin ether	0.5
Mineral oil	6.3
Lanolin alcohol	0.7
Oleic acid	2.7
Isocetyl stearate	10.0
Triethanolamine	1.3
Carbomer* 941	0.1
Glycerin	4.0
Preservative	0.4
Extensin solution from carrots ¹	5.0
20 Hydrolyzed extensin polypeptides ²	5.0
Water	qs 100.00

¹ 5% solution

² 5% peptide solids, "Vegagen*", Centerchem, Inc., Stamford,

CT

* denotes trade-mark

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Example 6

An oil in water moisturizing cream was made as follows:

	<u>w/w%</u>
Glyceryl stearate	5.0
Cetyl alcohol	2.0
Stearyl alcohol	2.0
Isopropyl stearate	4.0
Mineral oil	12.0
Polysorbate 60	1.0
10 Glycerin	8.0
Xanthan gum	0.25
Preservative	0.6
Extensin solution from carrots ¹	5.0
Hydrolyzed extensin polypeptides from carrots ²	5.0
Water	qs 100.00

¹ 5% solution

² Vegegen, Centerchem, Inc., Stamford, CT

Example 7

20 An oil/water cream makeup was made as follows:

	<u>w/w%</u>
Octyldodecyl stearyl stearate	4.0
Isocetyl stearate	1.0
Glyceryl stearate	6.0
Isostearic acid	2.0
Stearic acid	1.0

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	Ceteth 10	1.0
	Cyclomethicone	12.0
	Stearyl alcohol	1.5
	Nonionic surfactant mixture ¹	1.0
	Binders and thickeners	1.7
	Titanium dioxide	8.0
	Iron oxide	2.0
	Propylene glycol	3.0
	Triethanolamine	1.5
10	Preservatives	0.55
	Extensin solution from tomato ²	1.0
	Water	qs 100.00

¹ lecithin, polysorbate 20, sorbitanlaurate

² 1.5% solution

Example 9

A water/oil pigment emulsion makeup was made as follows:

	w/w%	
	Cyclomethicone	22.6 12.0
	Dimethicone	----- 5.0
20	Surfactant	16.0 20.0
	Laureth 7	0.5 -----
	Laureth 9	----- -----
	Bentone gellant	5.0 -----
	Iron oxides	3.1 2.4
	Titanium dioxide	12.0 8.5
	Talc	4.5 3.3
	Sodium chloride	2.0 2.0

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Propylene glycol	6.0	8.0
Preservative	0.5	0.5
Extensin solution from		
potato	0.5	0.5
Water		qs 100.0

Example 10

A water/oil pigment emulsion eyeshadow was made as follows:

	<u>w/w%</u>
Cyclomethicone	2.0
10 Dimethicone	5.0
Surfactant	20.0
Peg-7 C ₁₂₋₁₅ ether	0.5
Chromium oxide	6.2
Ultramarine blue	4.0
Titanium dioxide coated mica	6.0
Sodium chloride	2.0
Propylene glycol	8.0
Preservatives	0.3
Extensin solution from potato ¹	0.5
20 Water	qs 100.0

¹ 1% solution

Example 11

A protein shampoo was made as follows:

	<u>w/w%</u>
Ammonium lauryl sulfate	10.0
Cocamide diethanolamine	4.0

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Cocamidopropyl betaine	4.0
Ammonium chloride	0.8
Citric acid	0.1
Extensin solution from corn (1%)	1.0
Water	qs 100.0

Example 12

A creme rinse hair conditioner was made as follows:

	<u>w/w%</u>
Stearalkonium chloride	2.0
10 Cetyl alcohol	1.0
Stearyl alcohol	0.5
Ceteareth 20	2.0
Xanthan gum	0.5
Citric acid	0.3
Dimethicone	0.2
Extensin solution from corn (1%)	0.2
Water	qs 100.0

Example 13

20 A variety of extensin containing personal care products are made as follows:

	A	B	C	D
	<u>w/w%</u>			
Mineral oil	20.0	----	----	----
Beeswax	2.0	----	----	----
Polysorbate 40	8.0	----	----	----
Polysorbate 20	----	----	1.0	----
PEG 20 sorbitan				
beeswax	2.0	----	----	----

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	Stearic acid	10.0	----	----	----
	Petrolatum	4.0	----	----	----
	Sorbitol	5.0	----	----	----
	Ethyl alcohol	----	50.0	50.0	50.0
	Menthol	----	0.05	----	0.1
	Carbomer 940	----	0.75	----	----
	Triethanolamine	----	0.75	----	----
	Polysorbate 80/acetyl- lated lanolin				
10	alcohol/cetyl acetate	----	3.0	----	----
	Citric acid	----	----	----	2.0
	Glycerol	----	----	----	2.5
	Perfume	qs	8.0	8.0	0.5
	Preservative	qs	----	----	----
	Extensin solution from sycamore	0.5	0.1	0.1	0.1
	Water (qs 100)				

20 A is a cleansing cream, B is a clear fragrance, C is a toner, D
is an after shave.

Example 14

Two nail enamel preparations were made as follows:

	w/w%	
	<u>A</u>	<u>B</u>
Beeswax	12.0	-----
Lanolin, anhydrous	15.0	-----

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	Cocoa butter	8.0	-----
	Cetyl alcohol	3.0	-----
	Cholesterol	1.0	-----
	Mineral oil	30.0	-----
	Extensin solution from		
	carrots (0.2%)	5.0	0.5
	Preservative	qs	-----
	Perfume	qs	-----
	Water	qsl100	-----
10	Nitrocellulose	-----	15.00
	Toluene sulfonamide	-----	7.5
	Dibutyl phthalate	-----	3.75
	Butyl acetate	-----	29.35
	Ethyl alcohol	-----	6.4
	Butyl alcohol	-----	1.1
	Toluene	-----	36.40

While the invention has been described in connection with the preferred embodiment it is not intended to limit the scope of the invention to the particular form set forth, but, on the contrary, it is intended to cover such alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the appended claims.

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**THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE
PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:**

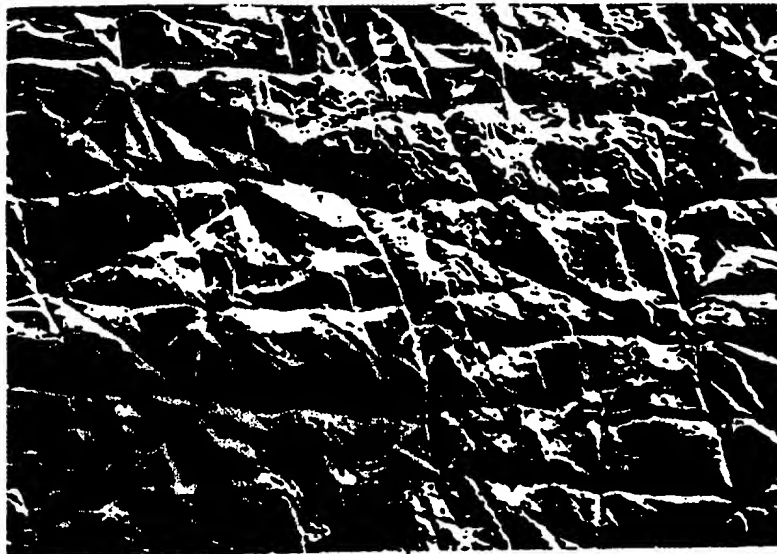
1. A cosmetic composition comprising a cosmetically effective amount of a substantially intact water soluble precursor form of extensin protein.
2. The composition of claim 1 comprising 0.01-30% extensin.
3. The composition of claim 1 wherein the extensin has a molecular weight of 100,000-150,000 daltons.
4. The composition of claim 3 wherein the cosmetic composition is a cream, lotion, gel, toner, fragrance splash, makeup, blush, eyeshadow, nail care preparation, aftershave, cleanser, shampoo or hair conditioner.
5. The composition of claim 3 comprising 0.1-10% extensin protein.
6. The composition of claim 5 comprising 0.5-5% extensin protein.
7. A pharmaceutical composition comprising from about 0.01% to about 30% of a substantially intact water soluble precursor form of extensin protein and a pharmaceutically acceptable carrier.
8. The composition of claim 7 comprising 0.01-30% extensin protein.
9. The composition of claim 8 which is a lotion, cream, ointment, gel, or solution.
10. The composition of claim 9 comprising 0.1-10% extensin protein.

11. The composition of claim 10 comprising 0.5-5% extensin protein.
12. A method for smoothing, moisturizing, and forming a film on human skin, nails or hair, comprising applying to said skin, nails or hair an effective amount of a substantially intact water soluble precursor form of extensin protein.
13. The method of claim 12 wherein the extensin protein is contained in a cosmetic.
14. The method of claim 12 wherein the extensin protein is contained in a pharmaceutical composition.
15. The method of claim 12 wherein the extensin protein is applied at least once every other day.
16. The method of claim 12 wherein the extensin protein is applied at least once a day.



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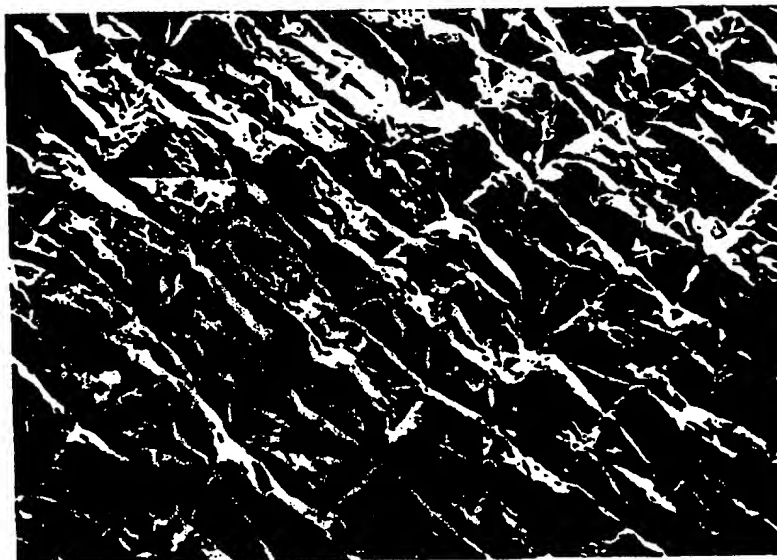
FIG. 1



NO TREATMENT

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FIG. 2



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